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Antitumor Activity of cis-Diamminedichloroplatinum (II) Against Human Tumor Xenografts Depends on Its Area Under the Curve in Nude Mice

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A pharmacodynamic analysis of cis-diamminedichloroplatinum(II) (DDP) was conducted using two human gastric cancer xenografts, SC-1-NU and MKN-45, and one human breast cancer xenograft, MX-1, grown serially in BALB/c nu/nu mice. DDP was administered intraperitoneally (ip) at a total dose of 5, 10, or 20 mg/kg in a schedule of q7d × 3 or (qd × 5) × 3. DDP was also administered ip to BALB/c +/? mice, whose plasma was used for the assay of total and free platinum by the atomic absorption method. A total dose of 20 mg/kg DDP seemed to be the maximum tolerated dose that was effective on MX-1 and SC-1-NU. When the totally administered doses were equivalent, the antitumor effects of the q7d × 3 and (qd × 5) × 3 schedules were similar to each other. The antitumor activity of DDP against MKN-45 was dependent on the total administered dose as well as the area under the curve of free and total platinum in the plasma. Side effects were significantly reduced using a schedule of (qd × 5) × 3 in terms of body and spleen weight loss when a total of 10 or 20 mg of DDP per kg was administered. These results suggest that DDP would be useful when administered using a daily schedule for obtaining the same antitumor activity as that of bolus injection but with reduced adverse effects.

KEY WORDS: **cisplatin, DDP, low-dose consecutive infusion therapy, human gastric cancer xenograft, pharmacodynamic study**

INTRODUCTION

Although cis-diamminedichloroplatinum(II) (DDP) has been used for treatment of human gastric cancers as a single bolus administration [1–3], its side effects, including nausea/vomiting, renal failure, and myelosuppression, have prevented the conventional use of this drug. However, low-dose daily administration of DDP has been used for genitourinary carcinomas [4,5] and head and neck cancer [6,7], and its application for gastric cancer is also being considered, although experimental studies of this method have not been fully conducted. We have already reported that the in vitro antitumor activity of DDP against the human gastric cancer cell lines MKN-45 and MKN-74 depends on its time × concentration product (AUC-vitro). In the present study, the antitumor activity and side effects of DDP were evaluated in vivo by comparing two different schedules of treatment with the same total dose, which was divided into 3 or 15 administrations, on human gastric cancer xenografts MKN-45 and SC-1-NU and the human breast cancer xenograft MX-1 in BALB/c nu/nu mice. A pharmacoki-

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TABLE I. Schedules of Treatment With DDP*

Tumor	Total dose of DDP (mg/kg)	Schedule
(1) MKN-45	20	a) q7d × 3 (on days 1, 8, 15)
	10	b) (qd × 5) × 3 (on days 1–5, 8–12, 15–19)
	5	
(2) SC-1-NU MX-1	20	a) q7d × 3 (on days 1, 8, 15)
		b) (qd × 5) × 3 (on days 1–5, 8–12, 15–19)

* Administered intraperitoneally.

netic study of platinum in blood was also conducted using BALB/c +/? immunocompetent littermates according to a different treatment method.

MATERIALS AND METHODS

Drug

cis-Diamminedichloroplatinum(II) (cisplatin : DDP) was purchased from Nippon Kayaku (Tokyo). DDP was administered intraperitoneally (ip) at a total dose of 5, 10, or 20 mg/kg, as shown in Table I. The treated mice consisted of two groups, which were given treatment schedules of q7d × 3 and (qd × 5) × 3, respectively. In the (q7d × 3) group, DDP was given on days 1, 8, and 15; in the (qd × 5) × 3 group, DDP was given on days 1 through 5, 8 through 12, and 15 through 19.

Human Tumor Xenografts

Two human gastric cancer xenografts, MKN-45 and SC-1-NU, and one human breast cancer xenograft, MX-1, were used for the experiments. MKN-45, a poorly differentiated gastric adenocarcinoma, was purchased from Immunological Biological Laboratory (Gunma, Japan) and was successfully transplanted into nude mice in our laboratory. SC-1-NU, also a poorly differentiated adenocarcinoma of the stomach, was established at the Second Department of Surgery (Nagoya University) and was kindly supplied by Dr. M. Yamauchi. MX-1 was established by Giovanella et al. [8] from cancerous tissue of a 29- year-old female patient with breast cancer and kindly supplied to our institute by Dr. K. Inoue (Cancer Chemotherapy Center, Tokyo), in 1979. These strains have been maintained in our experimental animal center by serial transfer in the subcutaneous tissue of BALB/c nu/nu mice.

A single tumor tissue fragment, 3 × 3 × 3 mm, was inoculated into the subcutaneous tissue bilaterally into the backs of nude mice under ether anesthesia using a trocar needle. The length and width of the tumor were measured by a sliding caliper three times weekly. The tumor weight was calculated using the formula: tumor weight (mg) = length (mm) × [width (mm)] ^{2}/2. Drug treatment was initiated when the tumor weight reached 100–300 mg. The relative mean tumor weight (RW) was calculated as RW = Wi/Wo, where Wi is the mean tumor weight at any given time and Wo is the mean tumor weight at the time of initial treatment. The antitumor effect was evaluated in terms of the lowest TRW/CRW value during the experiment, where TRW is the relative mean tumor weight of the treated group and CRW the relative mean tumor weight of the control group at any given time.

At the end of the experiments, the mice were sacrificed and the actual tumor weight and spleen weights were measured. The antitumor activity and the side effects in terms of spleen and body weights were evaluated as a ratio for the treated group relative to the control group (T/C ratio).

Statistical Analysis

Statistical analysis was performed according to the Student's *t*-test.

Mice

BALB/c nu/nu male nude mice were purchased from CLEA Japan (Tokyo). They were maintained under specific pathogen-free conditions using an Isorack and fed on sterile food and water ad libitum in our experimental animal center. Experiments were initiated when the mice reached a weight of 20–22 g and an age of 6–8 weeks.

The mice were weighed three times a week and the maximum body weight loss (B.W. loss) was calculated as a formula: B.W. loss = (initial mean body weight-maximally reduced mean body weight)/initial mean body weight (%) of the same group.

Pharmacokinetic Analysis

Pharmacokinetic analysis of DDP was conducted using tumor-free BALB/cA +/? mice (purchased from CLEA) maintained under conventional conditions at our animal center. DDP was administered intraperitoneally at a dose of 1.33 or 6.67 mg/kg, and four mice were sacrificed at 5, 30, 60, and 90 min, respectively, after drug administration by bleeding from the ocular vessels after ether-anesthesia, the blood being collected into a heparinized tube. After separation of the plasma by centrifugation at 3,000 rpm for 10 min, half of the plasma was stored for assay of total platinum at −20°C and the other half was additionally centrifuged at 3,000 rpm using the membrane of a Centrifree micropartition system (Amicon, Beverly, MA) and stored at −20°C for assay of free platinum. Total and free platinum was detected by the atomic absorption spectrophotometry and expressed as μg/ml [9]. The data were fitted to either a one- or two-compartment model, and either the Gauss-Newton, Damping Gauss-Newton or the Simplex method was used as the algorithm for calculation [10]. The area under the curves (AUCs) of

TABLE II. Antitumor Activities and Side Effects of DDP Against Human Cancer Xenografts

Tumor	Group	Mouse no.[a]	Tumor no.	T/C (%)[b] (relative)	Actual TW (mg)		T/C (%)[c] (actual)	T/C (%)[d] (S.W.)	B.W. loss[e]	
MKN-45	(1) DDP 20 mg/kg									
	a) control	5	10		615 ± 250	] $P = 0.08$				
	b) 3 times[f]	5	10	67.7	387 ± 301		62.9	57.7*	15.2*	
	c) 15 times[g]	5	10	55.8	336 ± 220	**	54.6	64.2*	15.1*	
	(2) DDP 10 mg/kg									
	a) control	5	9		856 ± 545					
	b) 3 times	5	9	59.3	698 ± 384		81.6	57.8*	13.6*	***
	c) 15 times	5	7	67.5	581 ± 502		67.9	95.8	2.1	
	(3) DDP 5 mg/kg									
	a) control	5	9		856 ± 545					
	b) 3 times	5	9	84.7	808 ± 743		94.0	100.8	3.0	
	c) 15 times	5	7	67.2	801 ± 360		93.9	102.8	1.6	
SC-1-NU	(1) DDP 20 mg/kg									
	a) control	4	6		3786 ± 909	*				
	b) 3 times	4	6	41.1	1611 ± 868		42.5	70.6	25.0*	
	c) 15 times	4	7	46.5	1374 ± 697		36.3	86.5	23.3*	
MX-1	(1) DDP 20 mg/kg									
	a) control	5	10		2650 ± 1542					
	b) 3 times	5	10	0	0		0	35.0*	21.9*	***
	c) 15 times	5	10	0	0		0	40.0*	13.6*	

[a] No deaths of mice were observed during the experiment.
[b] Lowest TRW/CRW ratio during the experiment.
[c] Actual tumor weight at the end of the experiment (T/C ratio).
[d] T/C ratio of spleen weight at the end of the experiment.
[e] Maximum body weight loss compared initial body weight (%) during the experiment.
[f] DDP was administered ip in a schedule of q7d × 3.
[g] DDP was administered ip in a schedule of (qd × 5) × 3.
* Statistically significant differences from control at $P < 0.005$.
** Statistically significant differences from control at $P < 0.05$.
*** Statistically significant differences at $P < 0.005$.

total and free platinum were calculated for the two groups, and thereby the total AUCs were calculated for each group.

RESULTS

Antitumor Activity

The antitumor activity of DDP against the human cancer xenografts MKN-45, SC-1-NU and MX-1 is shown in Table II. MX-1 was highly sensitive to DDP as reported elsewhere [11], and all the tumors disappeared completely at a total dose of 20 mg/kg under both the 3 and 15 times divided schedules. SC-1-NU was moderately sensitive to DDP, and the antitumor activity of DDP was almost equivalent under both the administration schedules in terms of relative mean tumor weight, for which T/C was 41.1% and 46.5%, respectively, and actual tumor weight, for which T/C was 42.5% and 36.3%, respectively. MKN-45 was less sensitive to DDP, and there were no statistically significant differences between the control and treated groups, even when a total of 20 mg of DDP per kg was administered. The antitumor activity of DDP against MKN-45 was dependent on the total administered dose from 5 to 20 mg/kg, and there were no significant differences between the 3 and 15 divided administration schedules in each total dose. Thus it was obvious that the antitumor activity of DDP was dependent on its total administered dose and that there were no differences in antitumor activity between the 3 and 15 divided administration schedules when the total administered doses were equivalent.

Toxicity

There were no deaths due to toxicity throughout the experiments. The toxicity of DDP according to the total dose and administration schedule was evaluated according to body and spleen weight (Table II). The body weight loss ranged from 13.6% to 25% when a total of 20 mg of DDP per kg was administered, suggesting that this total dose would be the maximum tolerated dose of DDP in BALB/cA nude mice. This body weight loss was statistically significant, whereas no body weight loss was observed when the total administered dose was 5 mg/kg. It was noteworthy that there was a significant body weight loss in mice treated with 10 mg DDP per kg in a schedule of three divided administrations, whereas no body weight loss was observed when the same total dose was given in a schedule of 15 divided administrations.

The spleen weights of MX-1- or MKN-45-bearing nude

TABLE III. Pharmacokinetics of DDP in BALB/cA +/? Mouse

	Dose[a] (mg/kg)	Cmax[b] (μg/ml)	AUC[c] (μg · h/ml)	cumulative AUC[d] (μg · h/ml)	T/C ratio[e] MKN-45	SC-1-NU
Free	1.33[f]	1.92	0.61	9.15	55.8	46.5
Platinum	6.67[g]	5.98	3.05	9.15	67.7	41.1
Total	1.33[f]	2.92	1.22	18.33	55.8	46.5
Platinum	6.67[g]	8.76	5.88	17.64	67.7	41.1

[a] Administered doses for BALB/cA +/? mouse intraperitoneally are shown as mg/kg.
[b] Peak drug concentration in the plasma.
[c] Area under the curve.
[d] Calculated cumulative AUC that would be given at a total dose of 20 mg/kg.
[e] Lowest TRW/CRW ratio during the experiment.
[f] Dose of 1.33 mg/kg DDP is once in a schedule of 15 times at a total dose of 20 mg/kg.
[g] Dose of 6.67 mg/kg DDP is once in a schedule of 3 times at a total dose of 20 mg/kg.

mice were significantly reduced relative to the control, when they were treated with a total dose of 20 mg/kg DDP in a schedule of 3 and 15 divided administrations. In MKN-45-bearing nude mice, there was no significant loss of spleen weight when a total of 5 mg of DDP per kg was administered, whereas the spleen weight was significantly reduced when a total of 10 mg DDP per kg was administered in a schedule of three divided doses, although there was no significant loss of spleen weight when the same amount of DDP was given in a schedule of 15 divided doses.

Pharmacokinetics of Platinum

The pharmacokinetics of platinum are shown in Table III. When 1.33 and 6.67 mg DDP per kg was administered, the maximum serum concentration (Cmax) of total platinum was 2.92 and 8.76 μg/ml, respectively, and the AUCs of total platinum were 1.22 and 5.88 μg · h/ml, respectively. The differences in dose, Cmax and AUC were 5, 3 and 4.8 times, respectively. This was almost the same for free platinum, suggesting that the protein-binding ratio did not differ for these administered doses. Since the total administered dose in the experiments to determine antitumor activity was 20 mg/kg, the cumulative AUC was calculated by multiplying AUC (1.33 mg/kg) × 15 and AUC (6.67 mg/kg) × 3, giving 18.33 and 17.64 μg · h/ml as total platinum for the schedules of (qd × 5) × 3 and q7d × 3, respectively, which were almost equivalent. This was also observed for free DDP. Since both of the administration methods produced almost the same antitumor activity on these xenografts, the antitumor activity of DDP appeared to depend on its AUC as well as the total administered dose, rather than Cmax.

DISCUSSION

Although improvements in early diagnosis and surgical treatment have markedly decreased mortality from gastric cancer in Japan [12], advanced gastric cancer still remains one of the most common fatal surgical malignancies in Japanese males [13]. DDP is one of the key drugs for the treatment of gastric cancer [1–3,14]. In our previous in vitro study, the antitumor activity of DDP was shown to depend on its concentration × time product (AUCvitro) against the gastric cancer cell lines MKN-45 and MKN-74.

In the present study, a pharmacodynamic analysis of DDP administration was conducted using human gastric and human breast carcinoma xenografts in nude mice to investigate the most appropriate method of DDP administration for human cancer by reducing the adverse effects of this drug. DDP gave almost equivalent antitumor activity when administered in a schedule of q7d × 3 or (qd × 5) × 3 against the sensitive xenograft MX-1, the moderately sensitive SC-1-NU, and the less sensitive MKN-45, and the antitumor activity against MKN-45 was found to depend on the total administered dose for both administration schedules. However, the toxicity of DDP was dependent on the administration schedule, the maximum body weight loss during the experiment, and the spleen weight loss at the end of the experiment being significantly different between the two administration schedules at a total dose of 10 mg/kg; the (qd × 5) × 3 method was tolerated better than the q7d × 3 method.

Pharmacokinetic analysis indicated that the cumulative AUCs were essentially identical to each other when the total administered doses were equivalent, although Cmax was elevated according to dose escalation. Furthermore, the protein-binding rates seemed to be stable under the different treatment schedules at the maximum tolerated total dose of DDP. This suggested that the antitumor activity of DDP was depended on its AUC, which is comparable with our previous findings in vitro that the antitumor effect of DDP depended on its concentration × time product rather than its concentration. Since the side effects of DDP were dependent on its Cmax, the (qd × 5) × 3 schedule was thought to be more useful than the (q7d) × 3 schedule when the same total doses were administered.

Our results are comparable with the report of Derwinko et al. [15], who found that continuous exposure to low-dose cisplatin resulted in a cell kill equivalent to short-term, high-dose exposure in cultured human lymphoma cells. Takahashi et al. [16] and Shimoyama [17] reported that the antitumor effect of DDP on G/S human gastric cancer xenografts in nude mice was better when a total dose of 9.1 mg/kg was administered bolus than for divided administration at the same dose, and they suggested that DDP would be classified as a Ib-type dose-dependent agent, according to Shimoyama's criteria. However, we have elucidated that the antitumor activity of DDP is dependent on its concentration × time product in vitro in the previous study and on the AUC in vivo in the present study.

This concept was also tested in a clinical trial by Jacobs et al. [7], who reported that the response rate to DDP administered at a dose of 50–130 mg/m^2 over 24 h for advanced head and neck cancer was equivalent to larger bolus doses, whereas the toxicity was reduced. Phase II trials suggested that bolus DDP at a dose of 200 mg/m^2 produced higher response rates than a conventional dose of 80–120 mg/m^2, although cumulative toxicity limited the treatment [18,19]. Forastiere et al. [6] reported that a total dose of 150 mg/m^2 of DDP administered for 5 days by continuous infusion or intermittent bolus against head and neck cancer was tolerable, so the spectrum of toxicity appears to be different because exposure to filterable platinum is significantly increased by continuous infusion. Thus the pharmacodynamics of DDP seem to be universal in different carcinomas, including those of the head and neck, ovary, esophagus, and also nonsmall cell lung and gastric carcinomas, in which intermittent administration will produce the same antitumor activity as bolus administration, whereas side effects are reduced.

A large bolus administration of DDP produces severe nausea/vomiting, nephrotoxicity, and bone marrow suppression, which require admission of the patients to prevent these side effects using antiemesis, hydration, and GCSF. The present study showed that the antitumor activity of DDP depends on its total administered dose and cumulative AUC, whereas its toxicity is related to the peak plasma concentration. These results suggest that DDP would be useful when administered in small divided doses, preserving its antitumor activity but reducing its side effects.

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